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GERMINATION AND FURTHER DEVELOPMENT OF THE EMBRYO OF ZEA MAYS SEPARATED FROM THE ENDOSPERM[1]

Demetrius Ion Andronescu

The Problem

The problem which the author proposes to investigate is a threefold one: (1) To ascertain the germination and further development of embryos separated from endosperms and also from scutella; (2) To find a proper medium for the germination of embryos which will furnish a substitute for the removed endosperms and scutella; and (3) To ascertain the effect in heredity, if any, of this traumatism.

Historical

Bonnet (2), as quoted by Stingl (12), is credited with being the first to study the germination of embryos detached from the cotyledons in his work with *Phaseolus multiflorus*.

Sachs (8), working also with *Phaseolus multiflorus* and with *Zea Mays*, pointed out that embryos when separated from their endosperms germinate poorly. He stated also in his *Physiology of Plants* (page 373) that the embryo during germination has a parasitic relation towards its endosperm, digesting and sucking up the latter through its absorption organ, the scutellum.

Gris (6) germinated embryos of *Mirabilis jalapa*, *Zea Mays*, and many other plants in moist sponges. He observed that the first stage of germination was based on the nutritive material found in the embryo itself, and only after the roots of maize attained a length of 3 cm. was he able to observe the appearance of sugar in the endosperm; he believed the scutellum to be only an absorption organ of the embryo.

Van Tieghem (13), working with seeds of *Helianthus annuus*, *Mirabilis jalapa*, and *Zea Mays* to ascertain (1) the dependence of the various parts of different organs of the embryo, and (2) the dependence of the embryo upon the endosperm; found that the plumule, radicle, and cotyledon, when separated and in a proper medium, but not the scutellum of the maize seed, are capable of independent growth and even of regeneration. Further, he found that the embryo of Mirabilis germinates easily without its endosperm, although the later is necessary in the advanced stage of germination for the development of the plumule. He demonstrated that the endosperm can be replaced by a paste made of triturated endosperm of the same seed or of seeds of different species, or by merely a paste made of starch to which have been added mineral nitrates and phosphates. He claimed therefore that the nutritive power of the endosperm does not depend upon its cellular organization. In another series of experiments, in which were involved *Mirabilis longiflora*, *Canna aurantiaca*, *Aucuba japonica*, and *Phoenix dactylifera*, the same author (14) concluded that "The endosperm of oleaginous and aleuronic seeds has a probe activity; it digests itself. . . . The starchy and cellulosic endosperms are, on the contrary, passive; they are digested by the embryos. . . . "

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[1] This article was read by title at the thirteenth annual meeting of the Botanical Society of America at Baltimore, Md., December 26–28, 1918. Owing to various circumstances the writer has been unable, up to the present time, to continue his investigations on this subject, but he has decided to publish these meager observations hoping that they may be of interest to others whose work is in the same or similar lines.

Bolciszwesky in 1876, as quoted by Brown and Morris (3), experimenting with embryos of oats, rye, and lupine which he germinated in moist soil, also concluded that the endosperm is not necessary to germination.

Brown and Morris (3) raised the question: "Is there any residual life in the cells of the endosperm?" By applying, or, to use the authors' own term, by "grafting" new living embryos to seeds of barley which had been killed by the action of heat, chloroform, and alcohol, these embryos developed almost normally, using these dead endosperms. These observations supplement the statement of Sachs that "The endosperm of the grasses is a dead storehouse of reserve material; therefore during the germinating period the life of the embryo is not parasitic but saprophytic."

Stingl (12) found that the endosperms of the seeds of wheat, rye, barley, and oats in many cases may be substituted one for another during the process of germination.

Dubard and Urbain (5) germinated embryos of seeds of barley, oats, and maize, without endosperm and cotyledon, in Knop's 0.5 percent nutrient solution. Some of their conclusions were: that the endosperm is not indispensable for the development of plants; that the endosperm is favorable in the first stage of germination; that the embryo possesses in itself the reserve material for its development; and that by mutilating the embryo in many cases a long vegetative development is obtained after which the plumule withers away, while the rootlets are very little advanced.

From the work of the above named authors the following conclusions may be drawn: (1) The endosperms of grasses are dead storehouses of reserve material at the disposal of the embryos; (2) That the endosperms are not essential for germination of the embryos.

We must bear in mind however that the authors quoted studied the germination of seeds and embryos without endosperms and cotyledons in different media and nutrient solutions, merely in their first stages of development, but that no one of them extended his study to the complete maturity of the plants developed from embryos without endosperm, together with the behavior of their offspring.

With regard to the anatomy and morphology of the maize seed, the writer would like to emphasize the fact that this question is by no means a settled one, especially that part of it which concerns the embryo. While in the majority of textbooks of botany the scutellum is described as "an haustorial suction organ of the embryo," it is not very clear where the scutellum ends and where the embryo begins; while the cotyledon of monocotyledonous plants has been reduced to a small region of a cell or cells to be seen only in the first stage of embryonic development.

Sargant and Robertson (10) claim that the scutellum of maize is distinguished by the presence of glands on its dorsal face and by the transfusion tissue connected with its vascular system. Both of these features are undoubtedly connected with its prolonged function as a suction organ.

Collins (4), after giving some of the opinions of other botanists, suggested that the plumule sheath is the coleoptyle, the region beneath the mesocotyl, and the part beneath this the radicle.

Worsdell (15) throws more light on the subject with his conclusions that the scutellum is the lamina of the cotyledon corresponding to that of the foliage leaf of the grass; the coleoptyle being part of the cotyledon, and the mesocotyl the elongated primary node.

In his experiments the present writer used kernels of *Zea indentata*, *Z. indurata*, *Z. zacharatta*, and *Z. everta*. The kernels were soaked for a few hours in warm water, and after removing the testa the scutella with the embryos were carefully detached from their endosperms.

Series of experiments were followed during the years 1917 and 1918, with embryos and scutella together, and also with embryos detached from the scutella. The results are summarized as follows:

GERMINATION IN DIFFERENT MEDIA OF EMBRYOS WITH SCUTELLA DETACHED FROM THEIR ENDOSPERMS

To test the vitality of embryos separated from endosperms, they were given a few hours, germination on water-soaked filter paper, and those that showed a beginning of germination were placed in different solutions in individual test tubes to complete the germination. As a check on this experiment, embryos were germinated also in sterilized sand, using the same solutions. The germination media were solutions, alone and in mixture, of sucrose, lactose, diastase, and agar, as well as Knop's 0.014 percent nutrient solution. The germination process was extended from 7 to 12 days at temperatures varying from 16° to 22° C.

The sucrose solutions proved to be the most satisfactory. Concentrations of 1 percent and 2 percent of sucrose produced the best germination and growth, as the following figures, which represent the average of ten plants in each case in a 7-days' germination test, show:

Embryos Germinated in	Average Length of Stem (Mesocotyl and Leaves)	Average Length of Principal Root
Sucrose 1 percent	104 mm.	80 mm.
" 2 percent	100 mm.	58 mm.
" 3 percent	84 mm.	96 mm.
" 5 percent	57 mm.	80 mm.
" 10 percent	46 mm.	83 mm.
Water alone	18 mm.	34 mm.

While each of the plants produced in solutions of 1 percent and 2 percent sucrose had one principal root and two or three secondary roots poorly developed, those in 3 percent, 5 percent, and 10 percent solutions had each one principal root and many secondary roots very strong and well developed. In solutions over 5 percent, the growth was slow but vigorous. The germinations in water alone were very poor. Worthy of notice also was the very poor germination of the embryos in solution of lactose and in mixtures of sucrose, lactose, and maltose, and the slow but vigorous germination in mixtures of sucrose and diastase. In all my experiments I took the 1 percent solution of sucrose to be the best medium for the germination of the embryos.

The next step was to study the difference between the germination of complete maize kernels and the germination of embryos. This experiment also was repeated many times with different varieties of maize, using both methods of germination, in test tubes and in sterilized sand. For the germination of kernels water alone was used as a medium, while for the embryos a 1 percent solution of sucrose was employed. The best and most uniform examples were obtained with kernels and embryos of the Yellow Dent variety, soaked over night and then allowed to germinate 7 days in sterilized sand, using water for kernels and 1 percent solution of sucrose for the embryos. The results were as follows:

	Length of Stem (Mesocotyl and Leaves)	Length of Principal Root
Kernels	100 mm.	200 mm.
Embryos	65 mm.	75 mm.

From this experiment we may observe that the embryos detached from their endosperms, when germinated, produced plants having exactly the same characteristics and features as those produced by using the whole kernels, the only difference being that they were smaller. Figure 4 better illustrates this fact.

GERMINATION IN DIFFERENT MEDIA OF EMBRYOS DETACHED FROM THEIR ENDOSPERMS AND SCUTELLA

From kernels soaked in water from 6 to 12 hours after the testa was removed, the scutella were removed with needles from the front and sides of the embryos, and then the tissues connecting the embryo with the scutellum were cut away with a dissecting knife. The embryos thus freed had a vermiform appearance and were formed, of course, of plumule and its sheath, mesocotyl, and radicle enclosed in its sheath. For the germination of these embryos small test tubes were used, and the embryos were suspended above the solution media by means of strips of filter paper, in one set of experiments, and by small rings of paraffine in another set. These experiments were checked in sterilized sand also.

While many appropriate media were used for germination, in no case was the writer able to obtain plants with stem and radicle over 3 cm. long. It is interesting to report that in the first two days of germination a maximum growth was noticed, and that afterward the growth was very slow, many embryos withering away in from 6 to 8 days. The stem produced was the expansion of the mesocotyl and the elongation of the plumule sheath in which the plumule could be seen to be of a light green color. One principal and two secondary roots, very poorly developed, were produced. In no case was the plumule able to split its sheath and push out leaves.

It was noticed that the withering and subsequent death of some embryos was, in the majority of cases, due to decay which developed at the point where the scutellum had been detached. The decay, starting in this region, soon encircled the embryo and separated the plumule from the radicle, which then could be pulled apart.

The following table will illustrate one set of these experiments:

The kernels of *Z. everta* used in this experiment were soaked for 12 hours in water, the embryos being then removed and germinated for 48 hours. The above figures represent the averages from 4 embryos in each case.

From this table it will be noticed that the best germinations were obtained in 1.5 percent solution of sucrose, in 1 percent sucrose solution with a trace of nutrient solution, and in distilled water also with a trace of nutrient solution. Secondary roots were produced only in the 3 percent and 5 percent sucrose solutions and in the nutrient solutions. An interesting fact was the lack of germination in mixtures of sucrose, lactose, and diastase, in which the embryos began to decay before any signs of germination appeared.

FURTHER GROWTH AND DEVELOPMENT OF EMBRYOS WITH SCUTELLA (ENDOSPERMS REMOVED) AS COMPARED WITH PLANTS GROWN FROM WHOLE KERNELS

Embryos and kernels of maize, after a germination of seven days in sterilized sand, the former being watered with a 1 percent sucrose solution, and the later with water alone, were transplanted to the garden for further development. The results of similar experiments in 1917 not having been uniform on account of transplantation, the experiments were repeated in

Germination Media	Concentration		Length of		Observations
	Simple	Mixture	Stem	Radicle	
			mm.	mm.	
Sucrose	0.5%		6	7	
Sucrose	1%		2	2	
Sucrose	1.5%		12	13	
Sucrose	2%		4	5	
Sucrose	3%		3	1	Vigorous stem.
Sucrose	5%		4	1	Vigorous stem.
Sucrose	1%				
Diastase	0.02%	1.02%	3	10	
Sucrose	1%				
Lactose	0.25%				
Diastase	0.25%	1.50%	0	0	No germination
Sucrose	1%				
Agar	0.25%	1.25%	3	4	
Sucrose	1%				
Gelatin	0.25%	1.25%	4	1	
Sucrose	1%				
Nutrient solution	Trace		10	13	
Sucrose	1%				
Nutrient solution	0.007%	1.007%	5	5	
Water					
Nutrient solution	Trace		12	11	
Nutrient solution	0.007%		2	2	
Nutrient solution	0.014%		9	5	Also developed secondary roots.

1918. The embryos with scutella were planted directly in the soil in a plot prepared for this purpose. One row of 16 embryos was planted on May 15, 1918, for each of the following strains of maize: Yellow Dent, White Dent, and White Flint; and rows of 16 kernels each were also planted for each of the same strains. All kernels germinated and the first leaves appeared at the surface of the soil in seven days, the embryos requiring eight days for the appearance of leaves. The difference became more marked during the following week, at the end of which time the plants produced from the kernels were almost twice the size of those produced from the embryos.

On June 2, two plants from each row were carefully removed from the soil and observations and measurements were made. The results in average are given in the following table:

These figures also confirm the statement made above, that plants developed from embryos show the same characteristics as those developed from kernels, although being inferior in size.

After June 3, however, the individual vigor and vitality of the plants grown from the embryos began to be asserted, and by the beginning of

	Plants Developed from					
	Yellow Dent		White Dent		White Flint	
	Kernel	Embryo	Kernel	Embryo	Kernel	Embryo
Leaves (number)	5	4	5	4	5	4
Length of mesocotyl and leaves	25 cm.	13 cm.	25 cm.	13 cm.	30 cm.	17 cm.
Length of mesocotyl	4 cm.	0.5 cm.	3 cm.	1 cm.	3 cm.	1 cm.
Length of principal root	25 cm.	8 cm.	20 cm.	12 cm.	30 cm.	15 cm.
Secondary roots (number)	4	3	4	4	4	3

July a few of the plants had attained a height as great as that of the plants developed from kernels.

The first tassels appeared in July 23 on one stalk in each row of both embryos and kernels in the flint strain. Generally the appearance of tassels and silk followed the individual vigor and development of stalks rather than the ordinary behavior of each of the strains. The tasseling period was extended from July 23 to August 10. The appearance of the silk and also the pollination, as well as the whole vegetative development, were hindered by the storm of August 8 and by the cold weather which followed for a few days afterward. On account of adverse weather conditions, the silk, like the tassels, appeared irregularly, and especially it was noticed that on a few stalks which were beaten down by the storm the silk appeared late, after the pollen had been entirely shed. All the plants were in the proterandrous condition.

During the time of pollination careful observations were made in regard to the vitality of the pollen. The writer was not able to detect any difference in size, shape, and especially in the vitality of the pollen produced by the plants developed from the embryos and by those produced from kernels. The vitality of the pollen was tested by artificial pollination and by germination of the pollen in a solution of 10 per cent sucrose and 0.75 percent agar (1).

Each stalk produced at least one ear, and there was only one barren stalk which appeared in the row of the Yellow Dent variety developed from the embryo.

On account of the cold weather the maize was harvested on September 15 without being perfectly matured, and the ears were incompletely developed. Some of the observations in regard to the number of internodes, length and diameter of stalks, etc., are given in the table following, the figures representing the average in each case taken from six typical stalks in each row:

From this table we observe that in each case the stalks developed from embryos were shorter than those developed from kernels, and this fact is explained by the decrease in the number of internodes which appeared in the stalks developed from embryos. Also the diameter of stalks was smaller in the plants developed from embryos. While there seemed to be no rule governing the node on which the ear appeared, it was noticed,

	Plants Developed from					
	Yellow Dent		White Dent		White Flint	
	Kernel	Embryo	Kernel	Embryo	Kernel	Embryo
Length of stalks (meters)	1.65	1.46	1.81	1.72	1.26	1.18
Internodes (number)	18	16	22	19	12	12
Diameter of stalks (cm.)	1.93	1.35	2	1.80	1.10	1.00
Ear developed at node	10	9	16	13	7	5

however, that in the stalks produced from embryos the ears appeared at a lower node than in other stalks, and that, while no ear was perfectly developed, those on the plants produced from the embryos were the best, the explanation being that pollination and fecundation occurred after the storm and cold weather of early August while in the case of the plants developed from kernels, pollination and fecundation occurred previous to and during the storm.

Discussion

This problem, as stated at the beginning of the article, is one which will require a study extending through several generations of maize in order to draw definite conclusions. The writer believes, however, that the results obtained from these preliminary experiments offer material for at least a discussion.

Sufficient proof is at hand to demonstrate that with maize, normal plants may be developed from embryos without endosperms. The germination of embryos with their endosperms removed seems to follow through exactly the same stages as those of the germination of whole seeds, except that the process is slower. The lack of the endosperm in germination does not so much influence the root system of the young plants as it produces a general retarding influence on the whole development.

The tendency toward a longer period of growth of plants developed from embryos with endosperms removed is reduced by the tendency of these plants to reduce their number of internodes.

The chemical composition of different anatomical parts of the maize kernel also must be considered. From the well-known work of Hopkins (7) the writer has compiled the following table:

	Percentage of Total in Kernel			
	Protein	Oil	Carbohydrates	Ash
Embryo	20.14	82.43	4.97	74.55
Endosperm	76.66	15.18	86.68	21.33
Testa	2.07	1.08	6.80	3.06
Tip Cap	1.14	0.69	1.56	1.06

From this table it will be seen that the embryo contains almost all the oil of the kernel and the largest percentage of minerals, while the endosperm contains almost all the carbohydrates and protein of the kernel. Theoretically, therefore, the slower germination of the embryos with endosperms removed is explained by the lack of carbohydrates. Practically, however, while vigorous plants can be produced from embryos the endosperms of which have been removed, the fact is demonstrated that the small percentage of protein and of carbohydrates which the embryo contains is sufficient to insure the germination and further development of the plants.

We can not deny, however, that the presence of endosperms is beneficial in the process of germination, as well as in the further development of the plants.

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EXPLANATION OF PLATE XLI

FIG. 1. *Zea indentata*, Yellow Dent variety. Kernels, median longitudinal section. × 2½.

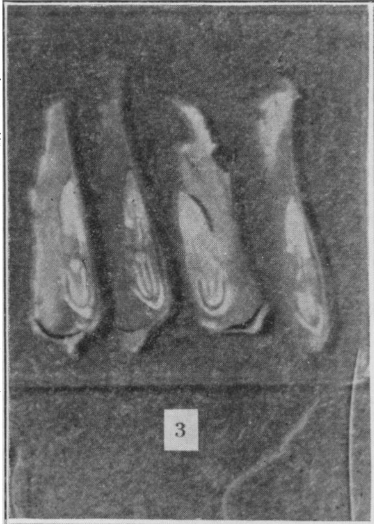
FIG. 2. *Z. indentata*, White Dent variety. Kernel after 10 hours' germination, fixed in alcohol; free-hand median longitudinal section, cleared in glycerine, mounted in glycerine-jelly. × 2½.

FIG. 3. *Z. indentata*, White Dent variety. Embryos after 8 hours' germination; free-hand median longitudinal section, cleared in chloral hydrate. Note the scutella and the embryos with plumule, mesocotyl, and hypocotyl or radicle. Note also the origin of dermatogen, periblem, and plerome in roots, and the first endogenous adventitious roots. × 2½.

FIG. 4. *Z. indentata*, Yellow Dent variety. Plants developed from whole kernel (left), and from embryo with the endosperm removed (right), after seven days' germination. 4/10 natural size.

FIG. 5. *Z. indentata*, White Dent variety. Mature plants developed from kernel (left), and from embryo with the endosperm removed (right).

FIG. 6. *Z. indentata*, White Dent variety. Ears produced by plant developed from whole kernel (left), and by plants developed from an embryo with the endosperm removed (right). ½ natural size.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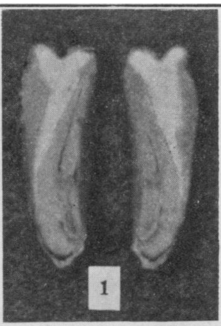
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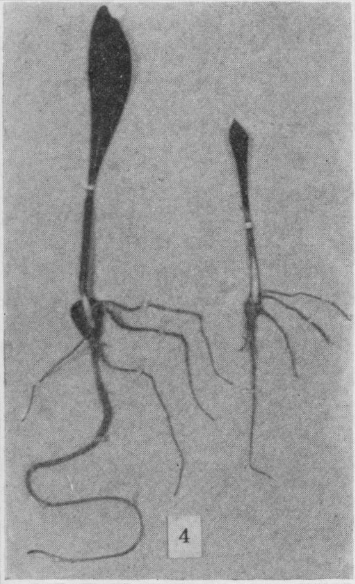
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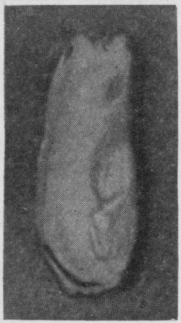
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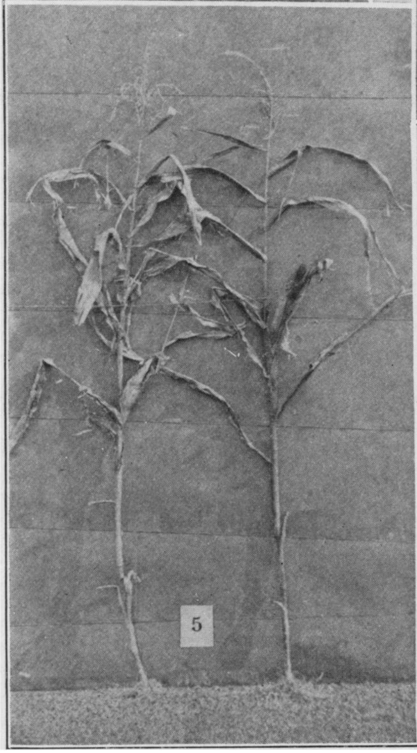
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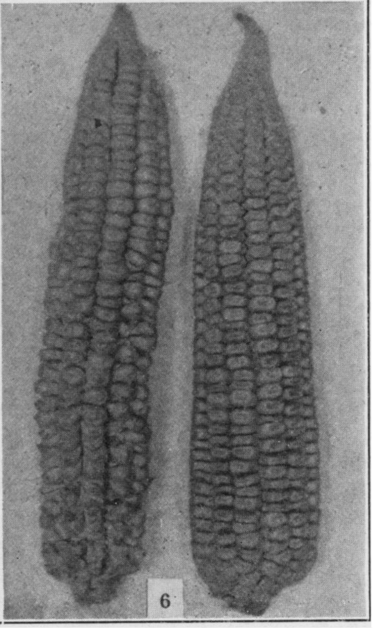
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ANDRONESCU: GERMINATION OF ZEA MAY.